

# Dual genitotropic human papillomavirus infections in genital warts

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## Abstract

**Background and methods**—We have carried out a prospective study of dual genitotropic human papillomavirus (HPV) infections by means of two different DNA detection methods in biopsy specimens obtained from patients who were examined for genital warts at the STD clinic of the School of Medicine in Seville, between January 1990 and December 1991.

**Results**—100 patients with a clinical diagnosis of condylomata acuminata were seen during the study period. DNA of the genitotropic HPV 6/11, 16/18 and 31/33/35 was detected by an in situ hybridisation method in 75 (77%) of the 98 evaluable samples; one of the genotypes tested in 59 (61%) samples, and two or more genotypes tested in the remaining 16 (15%) samples. In 21 (98%) of the 23 negative samples by in situ hybridisation, we were able to detect DNA of genital HPV using a polymerase chain reaction amplification method (PCR). Among the 34 samples where PCR was applied we confirmed the presence of two different HPV genotypes in eight samples.

**Conclusions**—The frequency of dual infections with human genitotropic papillomavirus in genital warts was 8%, although we believe that this rate should be higher as we have not used the PCR method in all of the samples.

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## Introduction

Condyloma acuminatum is the most common viral sexually transmitted disease in Western countries.<sup>1-8</sup> These genital warts are caused by human papillomavirus (HPV) infection of the anogenital squamous epithelium. Often, but not always, specific HPVs are associated with distinct clinical and histopathologic characteristics, such as HPV-6 or -11 with condylomata and HPV-16 with squamous-cell precancers and carcinomas.<sup>9-11</sup> These associations are not invariable, as HPV-6 has been found in anogenital squamous cell carcinomas and HPV-16 in some cutaneous condylomata.<sup>12</sup>

There is a paucity of data about dual infections with genital HPVs on condylomata acuminata, and there is not agreement about the rates of multiple infections (with genitotropic HPVs) ranging from none<sup>13</sup> to 13%.<sup>14-16</sup> The aims of the study were to know the prevalence of different genital HPV geno-

types in genital warts in Spain, to know the rate of dual infections in these patients, and finally to compare two methods for the detection of DNA HPV in genital warts and design a laboratory strategy for the diagnosis of genital HPV infections in a clinical setting.

## Material and methods

**Condyloma specimens.** Anogenital condyloma tissues were collected from 100 patients attending the STD Clinic of the School of Medicine in Seville. Condyloma acuminatum was diagnosed by gross appearance of the lesion and clinical history of the patient (table 1). The tissue available for laboratory analyses was usually a shave or punch biopsy. Half of the specimen was fixed in neutral 10% formalin for in situ hybridisation, while the other half was frozen at -70°C for PCR.

**In situ hybridisation (ISH).** Formalin-fixed tissue sections (10 µm thick) were hybridised with biotinylated HPV-6/11, or -16/18, or -31/33/35 in stringent conditions<sup>14</sup> using a commercial kit Enzo PathoGene (Enzo Diagnostics, Inc). Hybridised DNA was detected by sequential immunocytochemical reactions and visualised with a light microscope. Manufacturer's instructions were followed explicitly.

**Polymerase Chain Reaction (PCR).** The thawed specimens were minced and dissolved at 37°C in lysis buffer containing 50 mM Tris-HCl, pH 7.5, 0.5% SDS, 10 mM EDTA, 50 mM NaCl and 150 µg Proteinase K. After phenol and ethanol precipitation the DNA was pelleted by centrifugation. The pellet was dried and dissolved in 300 µl distilled water. PCR was performed as described previously.<sup>17</sup> The sequences for oligonucleotide consensus amplification and type-specific primers and probes were derived from conserved regions of the L1 open-reading frames of HPV-11, -16, -18; L2 of HPV-6 and E1 of HPV-33. These sequences have been described elsewhere.<sup>18-20</sup>

Table 1 Demographic characteristic of 98 patients and genital site of the lesions

Patients:	
Male	77
Female	21
Age	29.7, SD 9.4 (17-76 ys)
Location of the Lesions:	
External genitalia:	75
Male patients	57
Female patients	18
Perineal region:	
Male patients	20
Female patients	3
Number of Lesions:	6.02, SD 4.86 (1-25)

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Table 2 Sex distribution and prevalence of HPV genotypes as determined in situ hybridization method

Patients	HPV Genotype				None
	6/11	16/18	31/35	Mixed infection	
Males (n = 77)	44	1	2	13	17
Females (n = 21)	12	—	—	3	6
Total (n = 98)	56	1	2	16	23

The PCR reactions were performed in an automated thermocycler programmed for 32 cycles of DNA denaturation (95°C), primer annealing (55°C), and template extension (72°).<sup>21</sup> PCR products were transferred to nitrocellulosed and hybridised with specific (for 6, 11, 16, 18, and 33) oligometric probes. To avoid contamination, the PCR was performed in several separate work stations, the reagents were aliquoted from master mixes and the samples were added using piston-driven pipettes. Solution control without added DNA was included. Low positive control was DNA extracted from Hep-2 cells and negative control was DNA extracted from MRC-5 cells.

Results

*In situ hybridisation (ISH):* Of the 98 biopsy specimens evaluated, 75 (77%) showed evidence of infection with HPV types tested using the ISH method. Among these samples, we detected only one genotype in 59 (61%) samples while two or more genotypes were found in the remaining 16 (16%) (table 2). The genotype distribution found in the dual infections is summarised in table 3. The overall prevalence of the HPV-6/11,- 16/18 and -31/35 was 76%, 8% and 15% respectively. None of the women had HPV-16/18 infection, and in the three who had HPV-31/35 infection was associated with HPV-6/11. All but one of the HPV-16/18 infections detected in male patients were associated with other genotypes. HPV-31/35, infection was detected in 15 patients, but in thirteen of them it was associated to other genotypes.

*DNA amplification (PCR):* Of the 23 ISH-negative samples, 21 (98%) showed evidence of genitotropic HPV infection by means of PCR using a consensus primer. Sixteen of them were positive for HPV-6 type specific primer, one for HPV-11, three for HPV-6 and -11, and one for HPV-6 and -33. Of the 16 dual infections detected by ISH we have only been able to confirm four of them by PCR assays: two of them due to HPV-6 and -11, one by HPV-11 and -18, and one by HPV-11 and 33; and in three samples we were not able to

detect any of the genotypes tested two of them being positive with the consensus primer but type specific negative.

After combining the results of the two techniques, we demonstrated DNA of genitotropic HPV in 95 out of 98 samples (97%). DNA HPV-6/11 in 82 (84%), DNA HPV-16/18 in one (1%), DNA HPV-31/33/35 in two (2%), DNA consensus primer in two (2%), and eight (8%) dual infections.

Discussion

The present report is the result of a prospective study designed to determine the rates of the different genitotropic HPV genotypes in 100 patients diagnosed of condylomata acuminata in Spain. We found that HPV-6 and -11 genotypes are the most commonly associated with anogenital cutaneous condylomata<sup>12 13 22-24</sup> as previously described. Estimates of the proportion of condylomata positive for HPV-16/18 and HPV-31/33/35 range from none to 10%<sup>13-16</sup> and in our study were 2% and 4% respectively. We also confirm the limited sensitivity (77%) of the ISH method<sup>22 25-27</sup> and the poor specificity of the commercial kit we used due to the cross reactivity of the different probes, specially HPV types -16/18 and -31/33/35, because only four of the 16 dual infections detected by ISH could be confirmed by PCR assays. In addition, this method does not distinguish double infections if the types are in the same probing group (eg a -6 and -11 double infection).<sup>20</sup>

Rates of dual infections range from none or < 1%<sup>13-15</sup> to 13% or more;<sup>16-24</sup> our rate is 8% but it is difficult to compare the results as these investigations used different populations and methods, and generalisation among them cannot be made. Even though we believe that the rate of dual infections should be higher as far as using PCR, we have been able to detect four dual infections among the 23 negative samples by ISH.

We consider that in order to know the true prevalence of dual infection it should be convenient to apply a PCR assay that combines consensus and type-specific primers for the genitotropic HPVs, and the studies similar to ours can contribute to the knowledge of epidemiology and pathogenesis of HPV genital-anal infections.

Table 3 Genotype distribution found in the dual infections by in situ hybridisation method

Patients	HPV Genotype			
	6/11, 16/18	6/11, 31/35	16/18, 31/35	6/11, 16/18, 31/35
Males (n = 13)	3	6	1	3
Females (n = 3)	—	3	—	—
Total (n = 16)	3	9	1	3

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